

VOLATOLOMICS ANALYSIS OF LUNG AND COLON CANCER USING TERAHERTZ AND INFRARED SPECTROSCOPY

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A special dedication to my parents,
Arshad Mustapa & Rohana Hassan

To my beloved siblings,
Herman, Anis, Faiz, Hisyam & Tasya

To my beloved best friends,
Sarhan, Sya, Krik, Arep, Azani,
Razak, Fauzi, Syamil

Thank you for everything.

~ipsa scientia potestas est~

“KNOWLEDGE ITSELF IS POWER”

...with love and care

a.zulhilmi

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ABSTRACT

Terahertz and infrared spectroscopy are effective analytical spectroscopic techniques to identify and study the conformation and molecular interaction of the biomolecules. It has a huge potential in cancer diagnosis because these spectroscopies are non-invasive technique and do not require labelling for tissues and cells. Volatolomics analysis is a technique to analyse the volatile organic compounds (VOCs) emitted and released by human metabolites, which are not limited to breathe analysis. VOCs that are released by cancerous cells can be one of the bio-diagnostics techniques to diagnose cancer. Although studies on breath analysis have been widely carried out, the study of the volatolomics analysis by using Fourier transform infrared spectroscopy (FTIR) and Terahertz time-domain spectroscopy (THz-TDS) is still new. Both FTIR and THz-TDS instruments are installed with a gas cell sampling tools by absorption technique to analyse and detect the key species released from the VOCs. Lung cancer (NCL-H1299) and colon cancer (COLO320DM) cell lines are uas samples to identify the key species of each of the cancerous cells. The experiment has been verified and validated by comparing with control samples such as normal lung (MRC-5) cell lines, normal colon (CCD112CoN) cell lines, empty flask, air from the culture media and normal lab air. All the samples have been cultured into different sealed flasks for 24 to 120 hours, before the VOCs are collected and transferred into the gas cells to analyse using FTIR and THz-TDS. Hydrogen chloride and benzamide have been identified as key species for lung and colon cancer, respectively. These findings have been verified and validated by using residual gas analyser (RGA), gas chromatography – mass selective detector (GC-MSD), and confirmed by earlier literatures. A chemometric statistical analysis also has been applied to this study to extract the important information of the biochemical data from the VOCs with the greatest discriminative power and highest precision. These findings demonstrate the potential use of FTIR and THz-TDS as clinical tools through the volatolomics analysis. In addition, more work is needed if it is to be applied in clinical practice.

ABSTRAK

Spektroskopi terahertz dan inframerah merupakan teknik spektroskopik analitikal yang efektif dalam mengenalpasti dan mengkaji struktur interaksi molekul bagi sesuatu biomolekul. Ia merupakan potensi yang besar dalam proses diagnosis kanser kerana teknik spektroskopik ini adalah tidak invasif dan tidak memerlukan pelabelan untuk tisu dan sel. Analisis volatolomik pula merupakan satu teknik untuk menganalisis sebatian organik yang mudah meruap (VOCs) yang terhasil daripada proses metabolisme manusia, yang mana tidak terhad kepada analisis pernafasan sahaja. VOCs yang dihasilkan oleh sel kanser boleh menjadi salah satu teknik bio-diagnostik sel kanser. Walaupun kajian mengenai analisis pernafasan telah banyak dijalankan, tetapi kajian analisis volatolomik dengan menggunakan spektroskopi inframerah transformasi Fourier (FTIR) dan spektroskopi Terahertz domain masa (THz-TDS) masih baru. Kedua-dua instrument FTIR dan THz-TDS telah dipasangkan pada satu alat persampelan sel gas melalui teknik penyerapan untuk menganalisa dan mengesan spesies petunjuk daripada VOCs yang dilepaskan. Titisan sel-sel bagi kanser paru-paru (NCL-H1299) dan kanser kolon (COLO320DM) digunakan di dalam kajian ini untuk mengesan spesies petunjuk bagi setiap kanser. Ujikaji yang dijalankan telah diverifikasi dan divalidasi dengan membandingkan sampel terkawal seperti titisan sel paru-paru normal (MRC-5), sel kolon normal (CCD112CoN), udara kelalang kosong, udara daripada medium kultur dan udara persekitaran makmal. Semua sampel titisan sel telah dikultur melalui kelalang-kelalang yang kedap yang berbeza selama 24 jam hingga 120 jam, sebelum VOCs dikumpul dan dipindahkan ke sel-sel gas untuk dianalisis menggunakan FTIR dan THz-TDS. Hidrogen klorida dan benzamida telah dikenalpasti sebagai spesies petunjuk bagi kanser paru-paru dan kanser kolon. Penemuan ini telah diverifikasi dan divalidasi dengan menggunakan penganalisis gas sisa (RGA), kromatografi gas – pengesan jisim terpilih (GC-MSD) dan disahkan oleh literature terdahulu. Satu statistik analisis kemometri juga diterapkan untuk kajian ini bagi mengekstrak maklumat penting data biokimia daripada VOCs dengan kuasa diskriminatif terbesar dan kepersisan tertinggi. Hasil kajian ini menunjukkan potensi penggunaan FTIR dan THz-TDS sebagai peralatan klinikal menerusi analisis volatolomik. Di samping itu, kajian lanjut masih diperlukan jika ia ingin diaplikasikan di dalam amalan klinikal.

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LIST OF ABBREVIATIONS

THz-TDS	-	Terahertz time-domain spectroscopy
FTIR	-	Fourier transform infrared
VOCs	-	Volatile organic compounds
GC-MS	-	Gas chromatography – mass spectrometer
GC-MSD	-	Gas chromatography – mass selective detector
PCA	-	Principal component analysis
PLS	-	Partial least square
RGA	-	Residual Gas Analyser

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CHAPTER 1

INTRODUCTION

1.1 Overview

This introductory chapter illustrates the core of this study, including the background which stimulates this research work, the motivation for the focus of the work which highlighted the importance of the volatolomics analysis used in disease and cancer diagnosis, the problem statement, aim and objectives, scope, and significance of the study. This chapter is structured to introduce the huge potential of cancer detection through volatolomics analysis using Fourier transform infrared spectroscopic and Terahertz time-domain spectroscopy technique.

1.2 Background of the Study

Cancer is a major cause of mortality in this world with more than half a million deaths by the year 2013 in the United States alone, and the number of cancer cases are increasing every year, especially in the low and mid-income countries [1]. The imbalanced socio-economics from these countries led to lack of awareness, expertise and equipment to diagnose the cancer accurately, in short duration and cost effective manners.

In general, cancer can be diagnosed by invasive and non-invasive techniques. The invasive techniques, such as biopsy and endoscopy, involve making an incision in the body to gain access to the target area. The non-invasive techniques, such as imaging and laboratory testing, do not involve surgical procedures. There also are alternative techniques to detect and identify the cancer, such as breath analysis [2], chemometric analysis [3], bio-fluids analysis [4]s and others.

Fourier transform infrared spectroscopy (FTIR) and Terahertz time-domain spectroscopy (THz-TDS) based technique in cancerous tissue diagnostic are dependent on terahertz and infrared spectral analysis between healthy and cancerous tissue samples. However, spectrum broadening is the most challenging to determine [5,6]. The broadening character of the terahertz and infrared spectrum in particular recorded from liquid or solid samples can mask and also interfere with other cancerous tissue constituents, including water spectrum thus affecting the measurement resolution. Detection of volatile organic compounds (VOCs) produced by cancerous cell can be a better option as the terahertz and infrared spectrum of the gas exhibit less broadening character.

This approach allows for a better terahertz and infrared spectrum of key products of the VOCs to be identified [7,8]. This key product then can be used as a fingerprint for that particular cancer type. Other diagnostic tools have not been able to yield the biochemical information to identify the key species of the cancer. Even so, the current techniques to detect the cancer have many disadvantages, such as high cost, many procedure, contamination or side effects. The proposed techniques using FTIR and THz-TDS complement the other techniques in assisting the physician to diagnose the cancer effectively.

In this study, the development of cancer fingerprints uses FTIR and THz-TDS is to identify the key species of volatile organic compounds (VOCs). This approach will utilize the gas released from cancerous cell, which contains VOCs, and infrared light absorbance to monitor specific absorbance patterns which produced the following changes in chemical compound species: for example, 6-aldehydes, isoprene, n-butyl

acetate, and n-propyl propionate released by hepatocellular carcinoma cell using GC-MS [9].

1.3 Motivation of the Work

This study involves three key aspects: (i) developing a system to capture and measure the volatile organic compounds released by cancerous cells, (ii) measuring and identifying the key species, and (iii) verifying and validating the key species of the cancer. The following sub-section highlight the significance of the study and the importance of the current work.

1.3.1 Why is volatolomics analysis used in cancer diagnosis?

Volatolomics analysis is the examination of the volatile organic compounds (VOCs) released by all metabolites from living things for the presence of certain compounds to determine the presence of cancers or diseases of the human body. Volatolomics analysis is not limited to breath analysis, but it is covered the VOCs released from breath, sweat, skin, urine, faeces and vaginal secretions. Volatolomics analysis has huge potential in detection and identification of diseases and cancer diagnosis [10,11], especially when it is involved in the end products of cellular processes of the human as well as a non-destructive technique.

There are many advantages by using Volatolomics analysis to diagnose the cancerous or diseases samples compared to other conventional methods; for example, this analysis technique is a non-invasive method which may reduce the risks and be less harmful to the patient and personnel. Furthermore, the results can also analyze and appear immediately if we have an established database of the diseases.

1.3.2 Why should a volatolomics analysis system be developed?

This volatolomics analysis technique will assist physicians and medical experts to diagnose the cancer or other diseases effectively. The non-destructive sample collection technique will help some patients who have problems with conventional sampling and diagnostics technique.

1.4 Problem Statement

The VOCs released by cancerous cells can be one of the bio-diagnostics techniques to diagnose the cancer. Previous works on VOCs detection released by cancerous cells have been performed by using a few analytical instruments, such as gas chromatography – mass spectrometer (GS-MS), electronic nose, proton transfer reaction mass spectrometry (PTR-MS), selected ion flow tube – mass spectrometry (SIFT-MS) and ion mobility spectrometry (IMS). However, each of these analytical instruments had their limitations, such as the need to change the filters, cannot measure in real time and simultaneously, need sample preparations, not effective and time consuming. Fourier transform infrared spectroscopy (FTIR) and Terahertz time-domain spectroscopy (THz-TDS) with gas absorption sampling techniques can overcome this limitation. The key species from VOCs released by cancerous cells can be identified and obtained from the literatures and National Institute of Science and Technology (NIST) [12] databases. The key species will be verified with other gas recognition technique and validated with the literature. Then, the samples are analysed by using chemometric statistical analysis technique for the highest discrimination and precision of results.

1.5 Objectives

The aim of this study was to carry out a detailed study of the application of Fourier transform infrared spectroscopy (FTIR) and Terahertz time-domain spectroscopy (THz-TDS) as potential diagnostics tools for clinical use for detection of cancer through volatolomics analysis. The study shows the application of FTIR and THz-TDS as a method to characterise biochemical differences that detect and distinguish the volatile organic compounds released by cancerous cells. This is based on the FTIR and THz-TDS analytical instruments measurements and statistical analysis of lung and colon cancer cell lines as well as normal cell lines and control experiment.

The study aims to address the following objectives:

- a) To develop a technique to capture and measure the volatile organic compounds (VOCs) from lung and colon cancer cell lines
- b) To measure and identify the key species of the samples using Fourier transform infrared spectroscopy (FTIR) and Terahertz time-domain spectroscopy (THz-TDS)
- c) To analyse the samples using chemometric analysis and validate the key species

1.6 Scope of Study

This study is focused on identification of two types of cancer fingerprint, such as lung and colon cancer, through release and uptake of volatile organic compounds by cell lines by using Fourier transform infrared spectroscopy (FTIR) and Terahertz time-domain spectroscopy (THz-TDS).

This study consists of four parts of science and mathematics areas. Firstly, the concept of physics radiation of infrared and terahertz for detection of the samples. Second is biomedical samples, such as cancerous cell lines, to be detected and identified by the analytical instruments. Thirdly, the chemical compounds need to be identified as key species of the particular cancer from the volatile organic compounds (VOCs). Fourth is the mathematical statistical analysis of the sample for the highest discrimination and accuracy of the samples by using chemometric analysis.

1.7 Significance and Original Contributions of This Study

Detection and identification of cancer fingerprints are very important and have a high impact to the community. In this world of health and medical practice, fast techniques, cost-effective and accurate detection and identification of diseases and cancer is very crucial to assist the medical practitioner. The cancer also affects human health and causes the most human mortality every year, worldwide. In addition, research on identification of cancer fingerprint through release and uptake of volatile organic compounds using analytical spectroscopy instruments such as FTIR and THz-TDS is still new and not established yet.

The spectroscopic analytical instruments, such as FTIR and THz-TDS, with a combination of gas cell sampling tools and the analytical capabilities of this combination were demonstrated by simultaneous in vitro gas monitoring and detection of cancerous cells. Furthermore, assistance of chemometric analysis technique will provide the highest discriminative power and highest precision of the result.

The THz-TDS with gas analysis technique is a preliminary study to identify the cancer key species through volatile organic compounds (VOCs). The terahertz spectroscopy technique can capture the signal directly, simultaneously and effective

from the samples. All of the key species will be saved into a database and can be used as cancer identifier for patient using mobile THz-TDS – gas analyzer in the near future.

1.8 Thesis Structure and Organization

Chapter 2 of this thesis reviews the literature highlighting applications of FTIR and THz-TDS in biomedicine, cancer identification and volatolomics analysis. This includes detailed discussion on spectroscopic studies in various diseases in human tissues, cells and bio-fluids. This is followed by an outline of the methodology and materials used for analysis and identification of cancerous cells in Chapter 3. This chapter also covers system optimization and data analysis. The results are presented in Chapter 4 with discussion as the data was analysed. This chapter is divided into three sections, presenting and discussing results from a) cell lines analysis, b) detection of key species, and c) verification and validation. The conclusions drawn from the work will follow in Chapter 5 with suggestions for future work.

1.9 Summary

This chapter summarizes the foundation of this study to make sure this study will be achieved within the prescribed scope. Two types of cancerous cells will be investigated in this study, namely colon and lung cancer. Fourier transform infrared spectroscopy (FTIR) and Terahertz time-domain spectroscopy (THz-TDS) is used as analytical instruments. Chemometric analysis is also used to refine and to determine the highest discrimination and accuracy of the results.

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